Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

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Master's thesis/ Swedish University of Agricultural Sciences • 30hp
Agricultural Science Programme /Animal Science
Uppsala 2020

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Credits:	30 credits
Level:	Advanced
Course title:	
Course code:	EX0872
Programme/education:	Agricultural Science Programme/ Animal Science
Course coordinating department:	Department of Animal Breeding and Genetics
Place of publication:	
Year of publication:	2020
Cover picture:	Therese Schönborg
Online publication:	https://stud.epsilon.slu.se
Keywords:	Mountain Breed, Swedish Jersey, Swedish Red and white, Swedish Holstein, Capillary Electrophoresis, protein composition

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Abstract

The protein composition of milk samples from Norrland, Sweden was investigated by capillary electrophoresis, in order to evaluate the variation in protein profile from different breeds at different collection periods during the year. The protein profile is of importance for processability of the milk, and is known to be influenced partly by genetics. Bulk milk samples from 13 farms with Swedish Mountain Breed (SMB), Swedish Jersey (SJB), Swedish Red and White (SRB) and Swedish Holstein (SH) were analysed for content of αs_1-, αs_2-, β-, and κ-casein, as well as α-lactalalbumin and β-lactoglobulin. The samples were also analysed in regard to total protein content, fat content and casein micelle size (CMS). Sampling took place during three periods; November, February and September, and the breeds were compared between the "indoor period" (November and February) and "outdoor period" (September) as well as during the whole period of investigation. For analysis during the indoor versus outdoor period, SMB and SJB was set as one cluster. The analysis showed significant differences between the breeds during the period of investigation as well as between the indoor and outdoor period. During the period of investigation SJB had the highest inclusion of κ-casein, which was 3.29 percentage points higher compared to SRB and 1.67 percentage points higher than SH. SJB was found to have a significantly lower inclusion of β-casein compared to SH, SRB and SMB with a difference of 7.20, 5.92 and 5.64 percentage points respectively. SJB was found to have a significantly higher inclusion of β-lactoglobulin compared to SH by 1.24 percentage points. Significant differences was found between the clusters when the indoor and outdoor period was compared. The SMB/SJB cluster (indoor) and SMB/SJB (outdoor) was found to have a significantly higher inclusion of κ-casein by 2.31 and 2.99 percentage points respectively compared to SH (indoor). SH (indoor) was found to have a significantly higher inclusion of total β-casein compared to SMB/SJB (indoor) and SMB/SJB (outdoor) by 3.47 and 4.67 percentage points respectively. No significant differences regarding protein composition within any breed was found between the indoor and outdoor period. The fat content in SJB milk was found significantly higher compared to SMB, SRB and SH during the period of investigation by 2.44, 2.21 and 2.53 percentage points respectively. The CMS differed significantly between the breeds between the indoor and outdoor period. SH (outdoor) was found to have a significantly larger CMS compared to SH (indoor), SMB (indoor), SRB (indoor) and SJB (indoor) ($p=0.018$, $p=0.023$, $p=0.012$ and $p=0.034$ respectively). It was concluded that the protein profile varies between breeds, and that season of collection only had a limited effect on the milk- and protein composition. The small variance regarding season could partly depend on the low sample size. More extensive studies are needed in order to support the results.

Keywords: Swedish Mountain Breed, Swedish Jersey, Swedish Red and White, Swedish Holstein, Capillary Electrophoresis, milk protein, casein

Sammanfattning

Proteinsammansättningen i mjölkprover från Norrland, Sverige undersöktes med kapillär elektrofores för att utvärdera variationen i proteinprofilen från olika mjölkkoraser under olika perioder under året. Proteinsammansättningen är av betydelse för bearbetning och ystning av mjölken och påverkas delvis av genetik. Mjölktanksprover togs från 13 gårdar med Fjällkor (SMB), Svensk Jersey (SJB), Svensk röd och vit boskap (SRB) och Svensk Holstein (SH) och analyserades med avseende på innehåll av αs_1-, αs_2-, β- och κ- kasein, såväl som α -laktalalbumin och β -laktoglobulin. Proverna analyserades även med avseende på totalt proteininnehåll, fettinnehåll och kaseinmicellstorlek (CMS). Provtagning skedde under tre perioder; november, februari och september, och mjölkproverna jämfördes mellan "stallperioden" (november och februari) och "uteperioden" (september) samt under hela undersökningsperioden. För analys gällande stallperiod jämfört med uteperiod grupperades SMB och SJB till ett kluster. Analysen visade vissa signifikanta skillnader mellan raserna under undersökningsperioden samt mellan stallperioden och uteperioden. Under undersökningsperioden hade SJB det högsta innehållet av κ-kasein, vilket var 3,29 procentenheter högre än SRB och 1,67 procentenheter högre än SH. SJB visade sig ha ett signifikant lägre innehåll av β-kasein jämfört med SH, SRB och SMB med 7,20, 5,92 respektive 5,64 procentenheters skillnad. SJB hade ett signifikant högre innehåll av β-laktoglobulin jämfört med SH med 1.24 procentenheter. Signifikanta skillnader hittades mellan raserna när stallperioden och uteperioden jämfördes. SMB/SJB (stallperiod) och SMB/SJB (uteperiod) hade 2,31 respektive 2,99 procentenheter högre innehåll av κ-kasein jämfört med SH (stallperiod). SH (stallperiod) hade ett signifikant högre innehåll av totalt β-kasein jämfört med SMB/SJB (stallperiod) och SMB/SJB (uteperiod) med 3,47 respektive 4,67 procentenheter. Inga signifikanta skillnader gällande proteinsammansättning hittades inom någon ras när stallperioden och uteperioden jämfördes. Fettinnehållet i mjölk från SJB var signifikant högre jämfört med SMB, SRB och SH genom undersökningsperioden, med 2,44, 2,21 respektive 2,53 procentenheter. Signifikanta skillnader hittades gällande CMS vid jämförelse mellan raserna under stallperioden och uteperioden. SH (uteperiod) hade signifikant större CMS jämfört med SH (stallperiod), SMB (stallperiod), SRB (stallperiod) och SJB (stallperiod) ($p=0,018$, $p=0,023$, $p=0,012$ respektive $p=0,034$). Sammanfattningsvis varierar proteinprofilen mellan raser och säsong verkar endast ha en begränsad effekt på mjölk- och proteinsammansättningen. Den låga variansen beträffande säsong kan delvis bero på det låga antalet prover som analyserats. Mer omfattande studier behövs för att stödja resultaten.

Nyckelord: Fjällko, Svensk Jersey, Svensk röd och vit boskap, Svensk Holstein, Kapillär elektrofores, Mjölkprotein, Kasein

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1 Introduction

Dairy products from cows are used for human consumption all over the world due to its taste and nutritional value. It is also unique in the sense that milk is a versatile raw material when it comes to processing. Its original function of being predominantly produced for the calf's maturation has evolved into an important staple product meant for human consumption. The protein composition of milk is of great importance for the processing of dairy products. The breeds used for dairy production today are commonly selected for a high milk yield in order to achieve high productivity as well as economical profit. Milk composition and yield are known to be influenced by genetic factors, both related to breed and individual, as well as environmental factors such as maintenance, health of the cow, farm management and feed related matters (Walstra et al. 2006). Studies have shown that breeding for a high milk yield might have negative effects on protein composition, due to genetic correlations, hence changing the milk properties. The seasonal variation and time for milk collection might also have an impact on the final protein profile and the milk's processability (Lindmark-Månsson et al. 2003). In Sweden, the most commonly used breed is Swedish Holstein (SH), followed by Swedish Red and White (SRB). Minor breeds are Swedish Jersey (SJB) and Swedish Mountain Breed (SMB) (Växa 2019).

This masters' thesis is a part of a larger project, where a number of farms have been carefully characterised with respect to management and milk quality. The aim of the main project is to determine both farm- and milk factors importance for the cheese making properties. In the thesis the overall goal has been to evaluate the variation in protein profile from different breeds at different periods of collection in milk from Norrland, Sweden.

Milk samples from 18 farms were collected at three different occasions in February, September and November during a year. The farms varied in many aspects, such as breed, size of the herds, milking system and management.

Milk from the farms with SRB, SH, SMB and SJB (13 farms) were selected and compared regarding protein composition. It was also investigated how the protein composition varied between the indoor and outdoor period.

2 Literature review

2.1 The Swedish milk industry

In the Swedish agricultural sector the milk industry is the most economically valuable, representing around one fifth of the total value (SJV, 2019b). For the dairy farmers however, the economical pre-conditions are fluctuating, as the industry is very dependent on the milk price (SJV, 2019b). When delivering milk to the dairy, the payment to the farmer is partly based on the total concentration of fat and protein, however the protein composition is not considered. In Sweden, the number of dairy farms is decreasing rapidly (SJV, 2020). During 2018, the average dairy farm had 92 lactating cows. (SJV, 2020). During 2018 the total production reached 2 600 000 tonnes of milk, which equals a decrease of 2.6% compared to 2017. Between 2009 and 2018 the total amount of weighed in raw milk has decreased with approximately 6% in Sweden, but the amount of consumed milk in terms of dairy products has increased with around 16%. The gap has been compensated with an increase of imported milk and dairy products in order to meet the demand (SJV, 2019b).

During 2007, about a third of the total volume of the collected milk was used for cheese production in Scandinavia (Wedholm et al. 2006; SCB, 2007). One major aspect for the processability of milk is the protein composition, which is linked to the coagulation properties of the milk. The protein content in Swedish dairy milk varied significantly from year 1996 to 2009. In 1996 the protein content was found to be 3.37g/100g milk and in 2001 it had decreased to 3.28g/100g milk (Lindmark-Månsson 2003). In 2009 the protein content was found to have increased to 3.47g/100g milk (Lindmark-Månsson 2012). The properties of the proteins are of importance for the cheese making, both regarding the yield and quality of the cheese (Wedholm et al. 2006).

2.2 Breeds

2.2.1 SH

In Sweden, the most prevalent breed for dairy production is SH. The breed originates from the Swedish Friesian, and in 2018 56% of the total number of recorded dairy cows were of SH origin (Växa 2019). Characteristically SH cows are tall with long legs compared to the native breeds, with a mean weight of 650kg during the third parity (Koenen et al. 1999). When SH dairy cows are selected in favour of other breeds, it usually is due to their high milk yield. On average, an SH cow produces 10 349kg milk/year (Växa 2019). Generally, SH milk however contains less fat and protein compared to native breeds (see table 1).

Table 1 Breed mean values for milk from SH, SRB, SMB and SJB cows (Växa 2019)

	SH	**SRB**	**SMB**	**SJB**
Protein (g/100 g)	3.46	3.62	3.55	4.11
Fat (g/100g)	4.08	4.35	4.37	5.83
Kg milk/year	10 349	9 158	5 573	7 076
Kg ECM/year	10 491	9 707	5 891	9 036

Swedish Holstein (SH), Swedish Red and White (SRB), Mountain Breed (SMB) and Swedish Jersey (SJB), Energy corrected milk (ECM)

2.2.2 SRB

The second most used dairy breed in Sweden is the SRB. During 2018, 36% of the Swedish dairy cows were of the breed SRB (Växa 2019). The breed originates from the Scottish Ayrshire which was imported to Sweden in the 1830's. The Ayrshire and the native Swedish Red Pied were merged in 1927 and the breeding association SRB was founded (Korkman 1988). Compared to SH, SRB cows are a bit smaller and produce less milk. The average amount of produced raw milk per SRB cow and year was 9 158kg in 2018 (table 1). In 2019 the average percentage of protein in the milk was higher than in SH and Swedish Mountain Breed, but lower than in Jersey cows (Växa 2019).

2.2.3 SJB

The smallest dairy cow that is used in Swedish production is SJB. SJB are special in the sense that they produce milk with a high fat and protein content, but with a lower yield than SH and SRB (table 1). In 2018, SJB cows produced approximately 7 080kg milk/ cow and year (Växa 2019). Jersey cows are popular for crossbreeding with the commercial breeds in order to reach a heterosis effect. By crossbreeding with Jersey cows, the proportion of both milk fat and milk protein can be increased (Lopez-Villalobos et al, 2000).

2.2.4 SMB

The breeding association for Swedish Polled Cattle (SKB) was formed in 1938 in order to preserve the heritage of the two native, hornless breeds Swedish Red Polled and SMB (Korkman 1988). Despite the efforts, the SMB population has decreased rapidly during recent years. In 1965, 5.4% of all dairy cattle in Sweden was of SMB, and in 1980 the number had decreased to 0.7% (Växa 2019). During 2018, the number of SMB had decreased further to 0.3% of the Swedish dairy cows (Växa 2019). SMB are smaller than SH and SRB, reaching a weight around 400-450kg (Alskog et al. 1995). SMB cows have been found to have milk well suited for processing, as well as having a lower concentration of non-coagulating (NC) milk compared to milk from SRB cattle (Poulsen et al. 2017). It generally also has a higher prevalence of κ-caseins than the modern breeds, which has been connected to the coagulation properties of the milk which is important during cheese making (Wedholm et al. 2006). In comparison to SRB the percentage of protein and fat is similar, but the yield is lower and reaches in average 5 573kg milk/year (Växa 2019) (table 1).

2.3 Housing and management

The most prevalent indoor system for dairy cattle in Sweden is loose housing. Traditionally, however, tied up stables has been the common housing system and it is still in use. Since 2010, Swedish law prevents any new constructions with tied up stables in favour of loose housing for the animals to be able to perform their natural behaviours. Farms with already existing tied up stables are allowed to continue with the production, but no reconstructions are permitted (SJVFS 2019). The milking system also varies from automatic milking to using a milk parlour or tie stall milking

units. In 2018 the estimated number of Swedish farms using an automatic milking system was 31% (SJV 2019b).

During the indoor period it is possible to individualize the feed ration, which makes it easier to ensure that the cow consumes enough feed, both concentrate and forage. This is important in order to obtain high milk yield. The feed composition has been found to influence the milk fat composition, but only slight influences on the protein profile has been found (Walstra et al. 2006).

According to the law, all cattle in Sweden have to be on pasture during the summer months. Depending on where in the country the farm is located, the extent of the time on pasture varies. In the north of Sweden the pasture season can occur between the 1st of May and the 1st of October (SJV 2019a). During this time, the animals have to be kept outside for a minimum of 60 days, for at least six hours per day. Out of these 60 days, 30 of the days have to be between 1st of July and 31st of August (SJV 2019a). Many farmers, however, have a longer grazing period than this, also in the north (Bernes et al. 2019). For the remaining time of the day, the cows mostly are kept inside for milking and additional feed intake.

2.4 Milk composition

When making dairy products, the processability of the milk is of importance for the end result. The technological properties of the milk is to a large extent regulated by the protein composition (Dalgleish 1993). In addition to yield, the amount of protein and fat and the somatic cell count are directly linked to the economical profitability of the milk. The main components of milk are water, lactose, fat, protein, minerals and vitamins (Walstra et al. 2006). The milk composition depends on for example breed and individual variation, as well as on the stage of lactation and to some extent management and health of the cow (Walstra et al. 2006). The protein content is mostly determined by breed and genetic background of the individual cow (Dalgleish et al. 1993). As a result of breeding, the amount of protein in milk increased from 1980 and onwards, but in the recent years has remained stable around an average of 3.5% for Swedish cows (Växa 2019). The protein and fat content became especially important after year 2000, when concentration of protein and fat was added as parameters for the milk price. The fat content in Swedish milk has increased from 4.14% to 4.19% from 1980 to 2019 (Växa 2019).

The most dramatic changes in milk composition can be found 2-4 days after parturition when the cow produces colostrum. During the first days after partum the milk excreted from the cow contains a large amount of immunoglobulins (Ig), which decreases quickly with successive milking (Hurley & Theil 2011). The secretion of

protein dense colostrum is important for the development of calves' immune system, as they do not receive Ig through passive transportation of Ig (Hurley & Theil, 2011). The milk composition also changes if the cow has been struck by severe udder infection, mastitis (Walstra et al. 2006). Milk from cows suffering from mastitis has a higher than normal amount of somatic cells, mostly leukocytes, as a response to pathogenic bacteria. During mastitis the milk yield from the infected udder quarter, as well as the general condition of the cow, is also negatively affected.
To a smaller extent, this also occurs naturally at the end of a lactation period (Walstra et al. 2006).

2.4.1 Casein

In bovine milk, the casein family represents about 80% of the total amount of protein (Walstra et al. 2006). The caseins can be divided into the minor subgroups αs_1-casein (αs_1-CN), αs_2-casein (αs_2-CN), β-casein (β-CN) and κ-casein (κ-CN) (Dalgleish 1993; Farell et al. 2004). They are commonly in proportion α_{S1}-, α_{S2}-, β- and κ-CN, in assumed weight ratio of 4:1:4:1 (Fang et al. 2016). Each of the casein subgroups exists in various genetic variants (Walstra et al. 2006). To some extent these genetic variants can be connected to milk yield and composition (Gustavsson et al. 2014).

Caseins are special in the sense that they are hydrophilic as well as being nearly unable to denaturise. They exist in a relatively open structure due to their amino acid composition and are usually unaffected by heat treatment under 100°C (Walstra et al. 2006). This is due to them being phosphoproteins with a high proline content, which is of significance for their heat stability (Walstra et al. 2006). The caseins in milk exist in micelles instead of in serum as other milk proteins do. They have a negative net charge and consist of protein, water and salts. All caseins except κ-CN are able to bind to cations like calcium and magnesium (Walstra et al. 2006). The calcium binding property facilitates transportation of calcium from the cow to the calf (Nylander et al. 2014). When the milk reaches the stomach of the calf, the enzyme chymosin, found in rennet, is able to coagulate the milk. The coagulation is important for the nutrient retention in the stomach and improves digestibility (Fox et al. 2008).

The casein group is of great importance when turning milk into dairy products, as the proteins offer stability during processing and storage. The amount of casein in relation to the amount of total protein has a significant impact on the cheese yield. A study by Lindmark-Månsson (2003) showed that the amount of casein/100g milk decreased from 2.56g in 1996 to 2.50g in 2001. In a later study by Lindmark-Månsson (2012) the casein content in Swedish dairy milk was found to have increased significantly from 2.50g/100g milk in 2001 to 2.69g/100g milk in 2009. The number of caseins has remained stable between year 1996 and 2009. (Lindmark-Månsson

2012). Suggested reasons for the changes in casein content was increased milk yield, plasmin activity, feeding and breeding. A decrease in casein content is thought to have a negative effect on the cheese yield.

α-CN

In bovine milk, α-CN makes up a large part of the total amount of casein. About 40% of the casein fraction consist of αs_1-CN, and 10% consist of αs_2-CN (Farell et al. 2004). Both groups of α-CN are single chain polypeptides, and within each group they only differ depending on their degree of phosphorylation which depends on the number of available sites on specific amino acids (Farell et al. 2004). The αs_2-CN differ from the αs_1-CN as they have disulphide bonds as well as having additional phosphorus groups (Walstra et al. 2006). The αs_1-CN commonly exist as either αs_1-CN-8P or αs_1-CN-9P, while the αs_2-CN can be found as αs_2-CN-10P to 13P (Farell et al. 2004). Both versions exist within the casein micelles where they are carriers of predominantly Ca^{2+} ions (Nylander et al. 2014).

β-CN

The most hydrophobic casein is β-CN. The net charge is neutral, but the molecule has charged groups that are clearly distributed, with the head being polar and the tail being unpolar (Farell et al. 2004). The molecule is sensitive to plasmin, which is an enzyme with the ability to cleave the β-CN molecule (Farell et al. 2004). The cleaving produces the degradation products γ-CN and proteose peptones. The amount of degradation products depends for example on the age and temperature of the milk (Walstra et al. 1999). Parts of the β-CN molecule is soluble at low temperatures, which increases the viscosity of the milk (Walstra et al. 2006). At present, twelve variants of β-CN have been identified, with the most common ones being β-CN A1 and A2. At a molecular level, the two variants differ in their protein structure at amino acid position 67, where A1 milk has histidine and A2 has proline (Sodhi et al. 2012). The β-CN variant A1 has been associated with milk intolerance as well as cardiovascular diseases and type 1 diabetes, partly because of the release of a peptide called beta-casomorphin 7 (BCM7) during hydrolysis of the molecule (Laugesen & Elliot 2003). The A2 variant is supposedly more digestible for humans as it does not contain BCM7 (Pal et al. 2015). Jersey cows has been found to have a higher frequency of A2 milk compared for example Holstein cows (Merriman 2009). Milk with solely the A2 variant of β-CN has been promoted and sold as "A2-milk" due to the presumed health benefits compared to A1-milk, but the assumption was rejected by the European Food Safety Authority (EFSA) in 2009 (EFSA 2009). Another large fraction consists of β-CN-B, although it is less common than the β-CN-A variants. It has been found more commonly in well coagulating milk, which

indicates that it is a desired genotype for processing (Poulsen et al. 2017). The variant has been found in a larger concentration in Danish Jersey compared to SH and SRB (Gustavsson et al. 2014).

κ-CN

The only casein that is not able to bind calcium ions is κ-CN. This protein, that is located on the surface of the casein micelle, has the function of keeping the other caseins in place inside the micelle (Nylander et al. 2014). One end of the κ-CN is hydrophilic with a negative net charge, which is crucial for the micelle solubility in the milk. This is due to the level of glycosylation of κ-CN on the surface of the micelle and depends on how many of the nine glycosylation sites on the surface that are occupied by oligosaccharides (Lucey 2011). κ-CN can also be separated into different genetic variants. There are eleven variants found today, where variant A and B are the most prevalent (Farell et al. 2004). In most dairy breeds, except Jersey, the A variant has been found to be most common of the two (Ng-Kwai-Han & Grosclaude 2003).

For processing purposes, κ-CN is of great value. The molecule can be altered by the addition of either the rennet enzyme chymosine, acid or a mixture of acid and heat. When chymosine is added, the hydrophilic end of κ-CN, known as the "hair", is cut off by hydrolysis of a single peptide bond (Walstra 1990). During this action the κ-CN is converted into para-κ-CN and the cut off macropeptide dissolves. The κ-CN no longer acts as a stabilizer which causes the calcium ions to escape the micelle. This causes the caseins to precipitate and enables the calcium ions to form bridges which helps to form a cheese curd (Walstra et al. 2006; Nylander et al. 2014).

The casein micelle is also sensitive to changes in pH, especially around its isoelectric point (4.6) where it becomes insoluble (Walstra 1990). When acid is added to the milk, the net negative charge around the micelles changes, which disrupts the colloidal stability and causes the milk to aggregate (Walstra 2006). Acidification is industrially common for fermented milk products and for some cheeses (Fox & Brodkorb 2008).

2.4.2 Whey

Around 20% of the protein fraction in bovine milk consists of whey proteins, mainly α-lactalbumin (α-LA) and β-lactoglobuline (β-LG) (Farell et al. 2004). Together the fractions of β-LG and α-LA usually make up about 14% of the total amount of protein, and the other whey proteins makes up about 6% (Walstra et al. 1999). The remaining whey proteins consist of bovine serum albumin (BSA) and immunoglobulins (Dalgleish 1993). In contrast to caseins, the whey proteins are globular and

sensitive to heat treatment. They start to denaturise when heated above a temperature of 60°C, which is a property that is taken advantage of in for example yoghurt making (Nylander et al. 2014). When heated, the whey proteins bind to the casein micelle which promotes water retention ability and consequently the viscous texture of the final product (Nylander et al 2014). The remaining solution after removing caseins and fat from the milk, is called milk serum.

α-LA

The main function of α-LA is its involvement in lactose synthesis (Dalgleish 1992). The protein acts as a coenzyme, binding to β-1-4-galactosyltransferase in the mammary epithelial cell which enables lactose synthesis (Farell et al. 2004). The genetic variant α-LA-B can be found in most cattle breeds, but also variants A and C are present in some breeds (Farell et al. 2004). During the end of a lactation period, the level of α-LA in the milk decreases. This has also been correlated to a decrease in milk lactose levels (Farell et al. 2004) and milk yield.

β-LG

The largest part of the whey fraction in milk consists of β-LG. It can be found in several genetic variants, but in most breeds variant A and B are most common (Farell et al. 2004). The function of β-LG is not fully established, but it has been found to bind hydrophobic molecules and act as a transportation protein (Dalgleish 1992). When heated, the protein undergoes irreversible denaturation and attaches to other proteins containing cysteine or to other β-LG. This is done by creating disulphide bonds from the thiol group (Jørgensen et al. 2017; Dalgleish 1992).

BSA, immunoglobulins & lactoferrin

During the first days post-partum, when colostrum is produced by the cow, the number of immunoglobulins in the milk is especially high. Immunoglobulins, or antibodies, are crucial for the calf as they provide a defence against infections (Nylander et al. 2014). Immunoglobulins are produced by secretory cells and are commonly found in blood (Walstra et al. 1999).

BSA is found in all secretions of the body, including blood and milk (Farell et al. 2004). Its function is not certain, but like β-LG it is thought to act as a transportation protein, as well as having part in controlling osmotic pressure of blood and protect against free radicals (Dalgleish 1992).

Lactoferrin, a glycoprotein in the transferrin family, can be found in a variety of secretory fluids, including in milk and colostrum (Farell et al. 2004). The protein is able to bind to and transport iron ions, as well as having antibacterial properties (Farell et al. 2004). The concentration of lactoferrin has been found to increase as a

response to infection and it has also been suggested to enhance some functions of the immune system (Sánchez et al. 1992).

2.4.3 The casein micelle

As previously mentioned, the casein fractions in milk exist in micelles. The average casein micelle size (CMS) is around 150 nanometer in diameter (nm), but the size varies between 50-600 nm (Fox et al. 2008). In a study by Jørgensen et al. (2017) it was argued that the CMS has a connection to the κ-CN content, with smaller micelles (~129 nm) having a higher inclusion of κ-CN compared to bigger micelles (~183 nm). The study also concluded that an increased number of smaller micelles could be associated to increased gelation stability of the milk. Devold et al. (2000) found that the mean CMS was influenced by for example total casein content, κ-CN genotype and feeding regime. They found the mean CMS to be significantly larger in the milk from a group of Norwegian Red Cattle fed organically produced barley as supplement, compared to being fed conventionally produced concentrate. They could however not find significant differences regarding total protein and total casein in the milk depending on the different feeding regimes, but found a significant difference between different genetic variants of αs_1-CN (Devold et al. 2000). On the contrary, in a study by Glantz et al. (2010) no significant connection could be established between CMS and specific casein fractions.

2.5 Changes in milk and protein composition

There are multiple aspects to consider when trying to influence the milk composition, and the relationship between the feed and milk composition has been studied repeatedly. It has been found that by changing the feed, the fat content and fat composition can be altered (Nylander et al. 2014; Walstra et al. 2006). In a study by Johansson et al. (2013) it was found that intake of high protein concentrate increased the prevalence of fatty acids, and that a silage containing 17% crude protein had the ability to increase the content of alpha-linolenic acid (ALA) in the milk, compared to a silage with 13% crude protein. The authors also concluded that the protein content in milk could be slightly increased by feeding cows with high protein concentrate, but it only had a limited effect on the protein composition of the milk. However, the results regarding protein was not found to differ significantly between the feeding strategies.

In a study by Lindmark-Månsson et al. (2003), the composition of Swedish milk was investigated by analysing 94 parameters, among them total fat content, total protein content, total whey content, casein in relation to total protein and different

protein fractions. The changes were mainly linked to the milk fat content and milk composition, but to some extent also protein. It was found that total protein, casein and β-LG varied significantly between seasons. The changes were most pronounced in summer during pasture season, with peaks in September for total protein and whey proteins and a peak in November for total casein. The seasonal changes were thought to partly be due to different feeding strategies and qualities of feed. The authors could not connect the differences to any specific region in Sweden and rather found the geographical spread to vary with each analysed parameter.

When discussing milk protein content, the forage-to-concentrate ratio, as well as amount of dietary fibre, are important aspects. In most cases an increased amount of concentrate in the feed increases milk yield, and the protein content only changes slightly. It has been found possible to increase the total protein content in the milk by 0.4 percentage points if the proportion of forage is decreased by 10% (DM) (Jenkins & McGuire 2006). Although effective, the recommended forage proportion is to be kept above 40% in order to avoid metabolic diseases (Jenkins & McGuire 2006). On the contrary, other studies have proven it difficult to increase milk protein due to feeding strategies (Broderick 2003; Olmos Colmenero & Broderick 2006).

Studies regarding the impact of amount of protein in the feed, or source of protein, have only shown slight changes in milk protein content (Jenkins & McGuire 2006). In a review article by Bequette et al. (1998) the low impact of dietary protein transferred to milk protein, which is about 25-30%, was discussed. It was suggested to partly depend on restrictions of amino acid uptake in the mammary gland (Bequette et al. 1998).

When referring to cheese making properties, the protein composition and coagulation properties is commonly discussed. In a study by Poulsen et al. (2017) the protein profile of the native breeds SMB and Swedish Red Polled was compared to well-coagulating (WC) and non-coagulating (NC) SRB milk. It was concluded that the native breeds had a superior protein profile compared to SRB milk in regard to coagulation properties. The levels of αs_1-CN, αs_2-CN and α-LA had the highest impact on the coagulation properties (Poulsen et al. 2017). Earlier studies have pointed the concentration of κ-CN being the most important for coagulation (Wedholm et al. 2006).

2.6 Protein separation

2.6.1 Methods for protein separation

For successful protein separation and characterization, different methods can be used. One common analytical method is gel electrophoresis. With this method, the proteins are separated by their size and net charge by migrating through a gel (Berg et al. 2002). It is also possible to separate the proteins depending on their isoelectric point (pI) with for example a two-dimensional gel electrophoresis (2DGE). With this method the proteins are first separated due to their pI and secondly depending on their molecular weight. Although effective, the 2DGE is unable to separate proteins over 100 kilodalton (kDa). Another disadvantage is the low sensibility and ability to detect certain proteins. Both methods are inexpensive and simple to use, but require a lot of work (Weinberger 2000). High performance liquid chromatography (HPLC) is also commonly used. This method separates major proteins with high accuracy, but requires large sample sizes and has a lengthy analysing period.

2.6.2 Capillary electrophoresis

Another method that has been developed as an automated system of the gel electrophoresis is capillary electrophoresis (CE). The method is based on transportation of proteins through a silica infused capillary, filled with a buffer solution.
The separation of the molecules is due to an electric field where the ions migrate from one node to another with a high voltage power supply. The proteins migrate with different speed depending on their net charge, size and shape (Harris 2003).

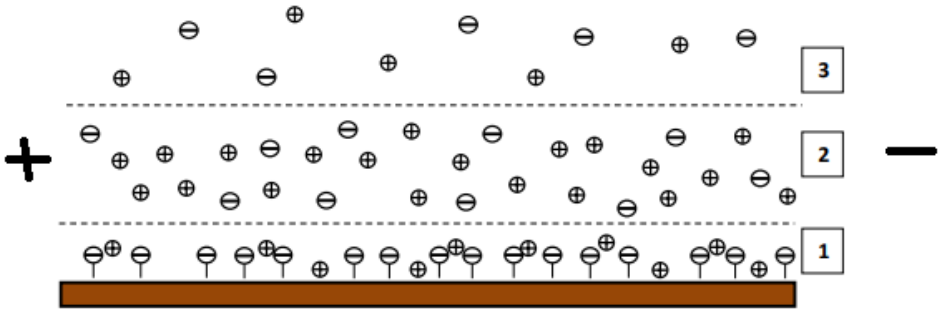

*Figure 1.*Schematic figure of the inside the capillary without added charge. 1) Silica coated surface with adsorbed cations. 2) Layer of a majority of anions, mobile phase. 3) Bulk solution, with a neutral charge.

The capillary is built up with a silica coated surface with a negative charge (figure 1). This charge is partly neutralised by different coatings or the addition of specific buffers, containing positively charged electrolytes that are absorbed by the silica layer (figure 1:1). Above the silica layer, the anions in the run buffer create a second layer with a mobile phase (figure 1:2). A third layer is created by the neutrally charged bulk solution (figure 1:3). The electric field that is implemented on the capillary causes the mobile phase to move from the anode towards the cathode, in a manner called electroosmosis (Harris 2003). When the sample is migrating through the capillary, the environment in the capillary is ideally uncharged. This enables separation between positively and negatively charged fractions (Harris 2003). One advantage with this method is that it does not use environmentally dangerous solvents e.g. acetonenitrile, which is used during HPLC.

3 Materials and methods

3.1 Milk sampling

Milk was collected from the tanks of 18 farms in Västerbotten county, northern Sweden, at three given periods in November 2017, February 2018 and September 2018. In each sampling period, there were three sampling occasions. Silo milk from the dairy plant was also collected at each occasion. The milk was defatted and stored in -80°C at the Swedish University of Agricultural Sciences (SLU) until analysing. In total, 242 bulk milk samples, including the samples of silo milk, were analysed for protein composition. In addition to the farm milk samples, control milk samples consisting of store bought, pasteurized milk were also analysed. The control milk was treated in accordance with the farm milk samples. Out of the 18 farms, 13 were chosen for further analyses in this study. 4 samples from the SRB cluster and 8 samples from the SH cluster were missing, giving 105 samples for statistical analysis. The selected farms were those that had a clear majority of either SRB, SH, SMB or SJB, with ≥ 70% of any given breed at all sampling occasions, resulting in a range from 72.7% to 100% of any given breed (table 2). The remaining five farms did not have a clear majority of one specific breed, and were therefore excluded from further statistical analysis. Out of the farms used in this master's thesis, three SH and one SRB farm kept the animals inside full time during the September sampling. The amount of feed fed inside also varied during September. All SH farms fed between 80-100% inside and the SRB farms fed 100% inside. The SMB farms fed 0% and 50% inside respectively and the SJB farm fed 10% inside. In addition to the milk samples, data concerning milk gross composition, casein micelle size, breed and sampling occasion were included. The silo tank samples were not statistically analysed in this study, but the complied results from the CE will be used in a future investigation regarding the proteolytic activity in relation to milk processability.

3.2 Analysis

3.2.1 Protein separation with CE

A sample buffer and a run buffer were prepared as follows. 6 M urea stock solution was prepared by adding 2g/100 ml water of ion exchange resin (AG® 501-X8 and Bio-Rex® MSZ 501(D), Mixed Bed Resin, Bio-Rad and 0.05% MHEC 3000. The sample buffer (pH 8.6 ±0.1) was prepared by mixing 167mM TRIS, 67mM EDTA, 42mM MOPS and 0.05% MHEC and was dissolved in the urea stock solution. For the run buffer (pH 3.0 ±0.1), 0.19 M monohydrate citric acid and 20mM trisodium citrate dehydrate was mixed. Both buffers were filtered through a 0.45µm filter paper (Durapore® membrane filters, Millipore, SE-171 28 Solna, Sweden) and kept in -20° until analysis. All chemicals were obtained from Sigma Aldrich.

The samples were analysed using a CE instrument (G-1600AX, Agilent Technologies Co., SE-164 94 Kista, Sweden) operated by Chemstation software (version A 10.02.). Before analysing, the skim-milk samples were incubated in a 45°C water bath for 2x15 minutes. In between the warming the samples were vortexed for 10 seconds. The sample solution was prepared by mixing 150µl of skimmed milk and 350µl of sample buffer in an Eppendorf tube. Before mixing, 2.6mg of 17mM DTT for each 350µl of sample buffer was added to the sample buffer. The finished sample solution was left in room temperature for >1h. The Eppendorf tubes with sample solution were centrifuged at 4°C at 10 000 rpm for 10 minutes before being defatted for second time using a cotton swab. The defatted sample solutions were filtered through a nylon membrane filter (Agilent captiva econo technologies filter, 0.45mm) before CE analyses. 30µl of the filtrate was transferred to a conic vial and run through the CE system. Left over filtrate was kept in -20°C.

The analyses were performed as described by Åkerstedt et al. (2012). An unfused silica standard capillary with 50µm inner diameter and 40 cm active length (Chrome Tech, SE-195 30, Märsta, Sweden) was used. Before analyses, the capillary was preconditioned with Milli-Q water for 10 minutes and left to rest for 5 minutes, followed by being flushed with run buffer for 20 minutes. The capillary was rinsed with Milli-Q water for another 10 minutes and finally with run buffer for 15 minutes. The separation of proteins was performed at 45°C with a linear voltage gradient from 0-25kV for three minutes. After three minutes, 25kV was constant. In between every sample, the capillary was flushed for three minutes with Milli-Q water before being flushed with run buffer for five minutes. The sample solutions were injected at the anode by pressure injection at 50mbar for seven seconds. The time frame for each sample to migrate through the capillary was found to be 45-60min.

3.2.2 Identification of peaks

After CE analysis, the baseline of the electropherograms was performed manually for all 242 samples (Figure 2). The identification was based on previously established electropherograms, identifying peaks of milk protein standards as α-LA, β-LG, αs_1-7-phosphate (αs_1-7P), αs_1-8-phosphate (αs_1-8P), αs_0-casein (αs_0-CN), αs_2-casein-10-phosphate (αs_2-CN-10P), αs_2-casein-11-phosphate (αs_2-CN-11P), αs_2-casein-12-phosphate (αs_2-CN-12P), κ-casein 1 (κ-CN_1), κ-casein 2 (κ-CN_2), κ-casein 3 (κ-CN_3), κ-casein 4 (κ-CN_4), κ-casein 5 (κ-CN_5), κ-casein 6 (κ-CN_6), β-casein A1 (β-CN A1), β-casein A2 (β-CN A2) and β-casein B (β-CN B) (Miralles et al. 2003). In addition to the listed peaks, total amount of β-CN, total CN and total whey proteins were calculated. The area under the peaks representing each protein, was calculated as a relative percentage of the whole protein profile detected.

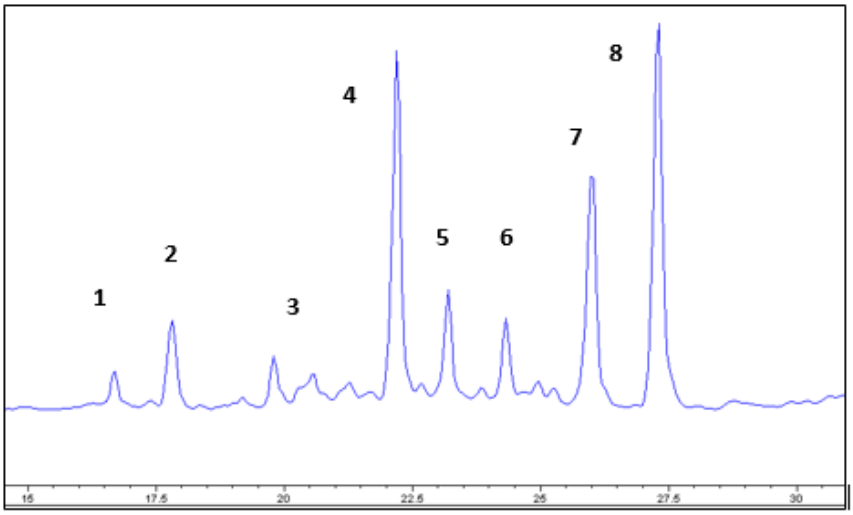

Figure 2. Representative electropherogram after capillary electrophoresis (CE) run, before baseline integration and peak identification. 1) α-lactalbumin, 2) β-lactoglobuline, 3) αs_2-casein, 4) αs_1-casein-8P, 5) αs_1-casein-9P, 6) κ-casein, 7) β-casein A1 and 8) β-casein A2.

3.3 Statistical analysis

3.3.1 Minitab

Values from the 13 chosen farms and control samples were statistically analysed in Minitab 18.1.0 (Minitab, Inc., USA) concerning the protein profile in relation to breed and production season. For the tests concerning the seasonal variation, trial from November and February were assumed as "indoor period" and compared to trial three (September) which is referred to as "outdoor period". The indoor period refers to a period where the cows were kept inside full time, and the outdoor period refers to a period where the cows were held partly on pasture, depending on the individual preconditions of the farms. For the analysis, one way ANOVA with Tukey pairwise comparisons with a significance level of 95% was used. The average CMS within each breed and change in CMS between the periods was compared, using one way ANOVA. To achieve method reproducibility, the coefficient of variation (CV) was determined for the control samples by calculating relative peak area, for all individual proteins.

Table 2 Data for the farms used for the statistical analysis

Farm	Predominant breed	Mean of the given breed (%)	Indoor/outdoor September	Inside feeding September (%)
1	SRB	85%	Indoor	100
2	SRB	90%	Outdoor	100
3	SH	100%	Indoor	100
4	SH	75%	Outdoor	85
5	SH	89%	Outdoor	100
6	SH	75%	Outdoor	95
7	SH	77%	Outdoor	95
8	SH	100%	Outdoor	100
9	SH	97%	Indoor	100
10	SH	83%	Indoor	100
11	SMB	100%	Outdoor	50
12	SMB	91%	Outdoor	0
13	SJB	80%	Outdoor	10

Swedish Red and White (SRB), Swedish Holstein (SH), Swedish Mountain Breed (SMB), Swedish Jersey (SJB). Number of farms = 13

4 Results

All results regarding casein content were verified by coefficient of variation (CV) values, which shows the reliance to the performance of the method. For all control samples except α-LA, the CV value reached about 10% or less (table 3).

Table 3 Descriptive data for control milk samples used in the capillary electrophoresis runs

Controls	Protein	N	Mean	SD	CV %
	α-LA	9	2.98	0.58	19.39
	β-LG	9	8.87	0.57	6.45
	αs_1-CN	9	32.79	0.90	2.74
	αs_2-CN	9	9.33	0.65	6.97
	κ-CN	9	7.34	0.76	10.31
	Tot β-CN	9	37.19	1.53	4.11
	Tot CN	9	86.66	1.04	1.20
	Tot Whey	9	11.84	1.11	9.33

α-lactalbumin (α-LA), β-lactoglobuline (β-LG), αs_1-casein (αs_1-CN), αs_2-casein (αs_2-CN), β-casein (β-CN) and κ-casein (κ-CN), total β-casein (Tot β-CN), total CN (Tot CN) and total whey protein (Tot whey), coefficient of variation (CV)

4.1 Clusters

For the statistical analyses regarding milk protein composition during seasonal changes, the farms with a majority of SMB and SJB were set as one cluster. This was done as these farms in general differ regarding the milk protein composition compared to the other commercial breeds SH and SRB, as well as to obtain a larger set of observations for the cluster. For this cluster three farms were included; two SMB farms and one SJB farm. The SRB cluster included two farms and the SH cluster included eight farms. For the statistical analyses regarding milk composition, the four breeds (SMB, SJB, SRB, SH) were kept separately.

4.2 Protein separation and variation in protein composition between breeds

In total 242 milk samples were analysed with CE, including the control samples. The relative percentages of α-LA, β-LG, αs_1-CN, αs_2-CN, β-CN and κ-CN were calculated for each sample. The samples from the 13 chosen farms and control samples were statistically analysed. When comparing the milk protein composition during the period of investigation, all four breeds were compared against each other (table 4). SJB was found to have a significantly higher inclusion of β-LG compared to SH by 1.24 percentage points (p=0.005). SJB milk was also found to contain a significantly higher amount of αs_2-CN compared to SRB, SH and SMB by 0.76, 1.22 and 1.3 percentage points (SJB-SH p=0.00, SJB-SMB p=0.00, SJB-SRB p=0.035). Regarding αs_1-CN, SJB had the highest mean inclusion with 33.35%, which was significantly higher than SMB by 2.01 percentage points (p=0.00). SMB also had a significantly lower inclusion compared to SH by 0.95 percentage points (p=0.014).

The amount of κ-CN in the milk differed significantly between the breeds with SJB having the highest mean value with 8.86%. This was 3.29 percentage points higher compared to SRB (p=0.001) and 3.5 percentage points higher compared to SH (p=0.00). SMB was found to have a significantly higher concentration of κ-CN compared to SH by 1.67 percentage points (p=0.01), but no significance was found between SJB and SMB, nor between SMB and SRB (p=0.105 and p=0.170).

SJB was found to contain a significantly lower amount of total β-CN compared to SH, SRB and SMB with differences of 7.2, 5.92 and 5.64 percentage points respectively (p=0.00 for all breed differences). Total CN, total whey and α-LA was not found to differ significantly between the breeds.

Table 4 Descriptive statistics for SRB, SH, SMB and SJB regarding the protein composition during the period of investigation. Means within a row with different superscript (a-c) differ significantly ($P < 0.05$)

	SRB			SMB			SH			SJB		
Protein	**N**	**Mean %**	**SD**	**N**	**Mean %**	**SD**	**N**	**Mean %**	**SD**	**N**	**Mean %**	**SD**
α-LA	14	2.33	0.81	18	2.26	0.73	64	2.16	0.80	9	2.09	0.68
β-LG	14	9.39 $^{a\,b}$	0.82	18	9.37 $^{a\,b}$	0.61	64	9.17 ^{b}	1.02	9	10.41 ^{a}	1.80
αs$_1$-CN	14	32.38 $^{a\,b}$	1.06	18	31.34 ^{b}	1.27	64	32.28 ^{a}	1.16	9	33.35 ^{a}	0.99
αs$_2$-CN	14	8.24 ^{b}	1.04	18	7.72 ^{b}	0.65	64	7.77 ^{b}	0.57	9	9.00 ^{a}	0.33
κ-CN	14	5.57 $^{b\,c}$	1.83	18	7.02 $^{a\,b}$	2.52	64	5.35 ^{c}	1.83	9	8.86 ^{a}	2.13
Tot β-CN	14	40.09 ^{a}	2.70	18	39.81 ^{a}	2.57	64	41.28 ^{a}	2.66	9	34.17 ^{b}	2.59
Tot CN	14	86.27	1.60	18	85.89	1.29	64	86.68	1.75	9	85.37	2.16
Tot Whey	14	11.72	1.45	18	11.63	1.28	64	11.33	1.75	9	12.51	2.30

Swedish Red and White (SRB), Swedish Holstein (SH), Swedish Mountain Breed (SMB), Swedish Jersey (SJB), α-lactalbumin (α-LA), β-lactoglobuline (β-LG), αs$_1$-casein (αs$_1$-CN), αs$_2$-casein (αs$_2$-CN), β-casein (β-CN) and κ-casein (κ-CN), total β-casein (Tot β-CN), total CN (Tot CN) and total whey protein (Tot whey)

4.3 Indoor versus outdoor period

For comparisons between milk protein composition during the indoor (November and February) and outdoor (September) period, SH, SRB and SMB/SJB was compared (table 5). Within each cluster, no significant difference was found between the outdoor (o) and indoor (i) period regarding any protein, but some significance was found when comparing between the clusters. The amount of κ-CN was found to be significantly higher in the SMB/SJB cluster compared to SH_i (table 5). Between SMB/SJB_i and SH_i there was a difference of 2.31 percentage points ($p=0.002$) and between SMB/SJB_o and SH_i 2.99 percentage points ($p=0.002$). The highest inclusion of κ-CN was found in the SMB/SJB_o cluster and the lowest within the SH_i cluster (table 5). SH_i was found to have the highest amount of total β-CN among all clusters with a mean of 41.8%. This was found to be significantly higher than SMB/SJB_i and SMB/SJB_o by 3.47 and 4.67 percentage points respectively ($p=0.001$ and $p=0.001$).

For α-LA, β-LG, αs_1-CN, αs_2-CN, total CN and total whey, no significant difference was found between the breeds regarding the indoor and outdoor period.

Table 5 Descriptive data for α-LA, β-LG, αs1-CN, αs2-CN, κ-CN, Tot β-CN, Tot CN and Tot Whey, concerning all breeds for the indoor and outdoor periods. Mean values are calculated as relative content of total protein (%). Means within a protein with different superscript (a-b) differ significantly ($P < 0.05$). SD - Standard Deviation is indicated

Period	Protein	Mean %	SD	Season	Protein	Mean %	SD	Season	Protein	Mean %	SD
SRB_i				**SH_i**				**SMB/ SJB_i**			
	α-LA	2.44	0.77		*α-LA*	2.06	0.78		*α-LA*	2.21	0.57
	β-LG	9.47	0.74		*β-LG*	9.05	1.06		*β-LG*	9.68	1.44
	αs_1-CN	32.17	1.17		*αs_1-CN*	32.20	1.16		*αs_1-CN*	31.68	1.3
	αs_2-CN	8.14	1.19		*αs_2-CN*	7.81	0.60		*αs_2-CN*	8.30	0.66
	κ-CN	5.65 $^{a\,b}$	1.62		*κ-CN*	5.09 ^{b}	1.92		*κ-CN*	7.41 ^{a}	2.43
	Tot β-CN	40.01$^{a\,b}$	2.15		*Tot β-CN*	41.80 ^{a}	2.60		*Tot β-CN*	38.33 ^{b}	3.74
	Tot CN	85.97	1.28		*Tot CN*	86.90	1.70		*Tot CN*	85.72	1.70
	Tot Whey	11.91	1.26		*Tot Whey*	11.12	1.75		*Tot Whey*	11.89	1.82
SRB_o				**SH_o**				**SMB/ SJB_o**			
	α-LA	2.05	0.96		*α-LA*	2.36	0.73		*α-LA*	2.19	0.96
	β-LG	9.20	1.11		*β-LG*	9.42	0.80		*β-LG*	9.79	0.65
	αs_1-CN	32.90	0.53		*αs_1-CN*	32.28	1.15		*αs_1-CN*	32.66	1.61
	αs_2-CN	8.48	0.52		*αs_2-CN*	7.65	0.52		*αs_2-CN*	7.84	1.07
	κ-CN	5.36 $^{a\,b}$	2.56		*κ-CN*	6.09 $^{a\,b}$	1.12		*κ-CN*	8.08 ^{a}	2.79
	Tot β-CN	40.27 $^{a\,b}$	4.18		*Tot β-CN*	40.08 $^{a\,b}$	2.05		*Tot β-CN*	37.13 ^{b}	3.72
	Tot CN	87.01	2.29		*Tot CN*	86.09	1.49		*Tot CN*	85.72	1.59
	Tot Whey	11.24	1.20		*Tot Whey*	11.78	1.49		*Tot Whey*	11.99	1.51

Swedish Red and White (SRB), Swedish Holstein (SH), Swedish Mountain Breed (SMB), Swedish Jersey (SJB), α-lactalbumin (α-LA), β-lactoglobuline (β-LG), αs1-casein (αs1-CN), αs2-casein (αs2-CN), β-casein (β-CN) and κ-casein (κ-CN), total β-casein (Tot β-CN), total CN (Tot CN) and total whey protein (Tot whey)

4.3.1 Variation in milk fat, milk protein and CMS

Regarding the total content of protein in the milk samples, the change during the period of investigation within and between the breeds was compared. No significant difference was found within either SRB, SH, SMB or SJB (figure 3). When comparing between the breeds, SJB differed from the other breeds, with a significantly higher inclusion of protein ranging from 0.95 (SJB-SRB) to 1.00 (SJB-SH) percentage points (p=0.00 for all) (table 6). When comparing between the indoor and outdoor period, SJB had a significantly higher amount of protein compared to the other breeds, ranging from 0.93 percentage points higher (SJB_i-SRB_o) to 1.09 percentage points higher (SJB_o-SMB_o) (p=0.00 for all) (table 6).

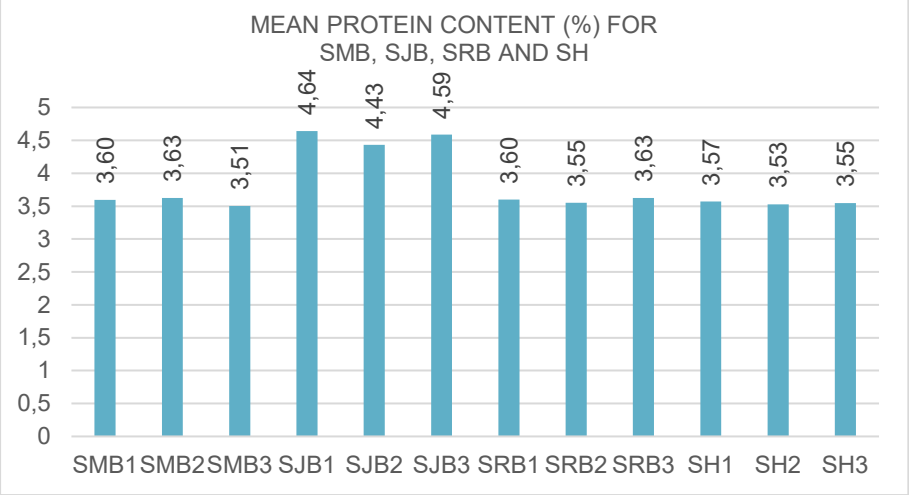

Figure 3. Mean protein content (%) for November (1), February (2) and September (3) for SMB, SJB, SRB and SH.
Abbreviations: Swedish Red and White (SRB), Swedish Holstein (SH), Swedish Mountain Breed (SMB) and Swedish Jersey (SJB)

The CMS was compared between the breeds and no significant difference was found during the period of investigation (table 5). When comparing each breed between periods, a significant difference was found between SH_o and SH_i where SH_o was found to have a larger CMS with 174.54nm compared to 158.43nm (p=0.018) (table 6). SH_o also had significantly higher CMS values compared to SMB_i, SRB_i and SJB_i with means mean values of 152.92nm, 144.46nm and 147.88nm respectively (p=0.023, p=0.012 and p=0.034 respectively) (table 7).

Table 6 Milk gross composition, mean values and standard deviation (SD) for SJB, SMB, SRB and SH during the period of investigation. Means within a row with different superscript (a-b) differ significantly
($P < 0.05$)

	SJB		SMB		SRB		SH	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Protein (%)	4.55 [a]	0.11	3.58 [b]	0.12	3.60 [b]	0.19	3.55 [b]	0.08
Fat (%)	6.83 [a]	0.21	4.39 [b]	0.30	4.62 [b]	0.49	4.29 [b]	0.21
CMS (nm)	149.36	4.58	159.00	13.00	155.01	9.43	164.29	13.08

Swedish Red and White (SRB), Swedish Holstein (SH), Swedish Mountain Breed (SMB), Swedish Jersey (SJB), casein micelle size (CMS)

Table 7 Milk gross composition, mean values for SJB, SMB, SRB and SH for indoor (i) and outdoor (o) season. Means within a parameter with different superscript (a-c) differ significantly ($P < 0.05$)

	SJB_i		SJB_o		SMB_i		SMB_o	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Protein (%)	4.54 [a]	0.15	4.59 [a]	-[1]	3.61 [b]	0.08	3.51 [b]	0.21
Fat (%)	6.86 [a]	0.28	6.76 [a]	-[1]	4.51 [b c]	0.15	4.16 [b c]	0.47
CMS (nm)	147.88 [b]	5.36	152.30 [a b]	-[1]	152.92 [b]	8.04	171.20 [a b]	14.40
	SRB_i		SRB_o		SH_i		SH_o	
Protein (%)	3.58 [b]	0.11	3.63 [b]	0.33	3.55 [b]	0.08	3.55 [b]	0.09
Fat (%)	4.80 [b]	0.22	4.36 [b c]	0.81	4.37 [b c]	0.16	4.16 [c]	0.24
CMS (nm)	144.46 [b]	7.25	160.29 [a b]	4.38	158.43 [b]	11.13	174.54 [a]	9.75

Swedish Red and White (SRB), Swedish Holstein (SH), Swedish Mountain Breed (SMB), Swedish Jersey (SJB), casein micelle size (CMS)

[1] *Only one sample available*

The amount of fat was also statistically analysed. When comparing the average inclusion during the period of investigation, no significant difference was found between SMB, SRB and SH. SJB had a significantly higher amount of fat compared to the other breeds (table 6). The fat content in SJB milk was 2.44, 2.21 and 2.53 percentage points higher compared to for SMB, SRB and SH ($p=0.00$ for all) (table 6). When comparing between periods, the highest amount of fat was found in the SJB_i milk with an average of 6.86% (table 7). No significant difference was found compared to SJB_o. SJB_i and SJB_o had significantly higher amounts of fat compared to all other groups (SMB_i, SMB_o, SRB_i, SMB_o, SH_i and SH_o), ranging from 2.21 (SJB_o-SRB_i) to 2.71 percentage points higher (SJB_i-SMB_o) ($p=0.00$ for all). SRB_i was found to have a significantly higher amount of fat compared to SH_o, with a difference of 0.64 percent points ($p=0.021$).

5 Discussion

5.1.1 Method

During preparation of the milk sample filtrates, DTT was added as a reducing agent in order to denature proteins by reducing their disulfide bonds to allow for better separation of them during electrophoresis. At some occasions, filtrates were prepared in advance and left in -80°C storage for 1-3 days before use. Left-over filtrates were also kept in -80°C as a precaution in case an electropherogram did not come out as expected, in which case the same filtrate could be run though the CE once again. During the drawing of the baseline in the electropherograms, it was found that the proteins in the filtrates do indeed denaturise exponentially with time, as some electropherograms derived from frozen filtrates did not display any or only some of the serum proteins. This could be the reason for the low percentage of whey proteins found in the control samples. The mean value of the whey proteins through the runs was found to be 11.8% (data not shown), which is low compared to the standard 20%. The lower percentage of the whey proteins could also be explained by the fact that only two whey proteins α-LA and β-LG are detectable by the method of CE. Other whey proteins as e.g. BSA did not contribute to the total whey proteins number.

When comparing the breeds with each other, the ratio between total CN and total whey in the milk samples was not found to differ significantly. The casein composition, however, did as expected vary between the breeds. The most notable differences during the period of investigation were connected to κ-CN and β-CN. The κ-CN concentration in SJB milk was found to be the highest, but with no significant difference compared to SMB. The concentration of κ-CN in SJB milk was however significantly higher compared to SRB ($p=0.001$ and SH ($p=0.00$). Wedholm et al. (2006) found milk with low amounts of κ-CN to be connected to poorly coagulating

milk. The study also concluded that milk with high contents of αs_1-CN, β-CN, and κ-CN in relation to total CN was suitable for processing. According to the results of the present study, SJB and SMB seems to be superior breeds in regards to κ-CN content and supposedly also processing.

The content of β-CN in the SJB milk was found to be significantly lower than in the other breeds (p=0.00). This is in agreement with Gustavsson et al. (2014) where a lower prevalence of β-CN in SJB was observed in milk from Danish Holstein compared to SRB. The low concentration could also be a result of the SJB cluster only including one farm and the low prevalence could be explained by the factors on that individual farm. The results might therefore not represent all genetic variants of each protein, and might not be representative for the breed as a whole.

5.1.2 Indoor versus outdoor period

When comparing the protein composition between the indoor and outdoor period no significance was found within the SRB, SH and SMB/SJB clusters. This corresponds with the study by Lindmark-Månsson et al. (2003), where it was concluded that the protein composition is rather stable throughout the year. However, when investigating the prevalence of each protein between the indoor and outdoor period, the results showed that both SMB/SJB_i and SMB/SJB_o had significantly higher values of κ-CN, compared to SH_i (p=0.002). Tot β-CN was found to be significantly higher for SH_i compared to SMB/SJB_i and SMB/SJB_o (p=0.001). Although significant, the results most probably depend on the genetic differences between the breeds as opposed to the change in periods. Different feeding regimes between the periods might have a slight impact on the milk composition, but no clear connection could be seen between protein profile and the periods. These results are in agreement with earlier studies, suggesting that it is difficult to affect the protein composition through dietary changes (Johansson et al. 2013; Jenkins & McGuire 2006). In the study by Lindmark-Månsson et al. (2003), changes in milk composition were more notable during the summer months, but no such conclusions could be drawn in the present study. However, three SH farms and one SRB farm kept the cows indoor full time for the September sampling. Most of the remaining farms also fed the majority of the feed inside at the time, which might have influenced the results and might be a reason for the small changes between the outdoor and indoor period.

5.1.3 Milk composition

When comparing the average of all sampling occasions, milk from SH farms was found to have the largest CMS and SJB was found to have the lowest (table 6) but

the difference was not significant. When comparing between breeds and periods, the highest values were found in milk from SH_o samples and the lowest in SRB_i samples (table 7). In the study by Jørgensen et al. (2017) it was suggested that small micelles (~129 nm) in the milk might help to increase gel stability, as smaller micelles were found to contain more κ-CN than larger ones. The values from the outdoor period found the CMS to be larger within each breed compared to the indoor period, suggesting that the CMS could depend partly on season with changes in feed composition whilst on pasture. However, the only significant difference was found within the SH cluster with SH_o having larger micelles than SH_i ($p=0.018$). The CMS has also been thought to depend on total content of casein and inclusion of κ-CN. In the study by Wedholm et al. (2006) the κ-CN content was found to have a positive correlation with well-coagulating milk, which indicates that the concentration of κ-CN could have an impact on the milk clotting properties. The CMS was significantly higher for the SH_o group compared to SRB_i, SMB_i and SJB_i ($p=0.012$, $p=0.023$ and $p=0.034$). This, in addition to having the lowest inclusion of κ-CN, suggests that milk from SH might be less appropriate for processing.

Both SMB and SJB produce less milk compared to SH and SRB. Milk yield is an important aspect for the economic stability of the farmers, which makes it an important trait for breeding. However, as the farmer is economically dependent also on the levels of fat and protein in the milk for the final milk price, it is of importance to breed for dairy cattle with maintained yield but not to compromise with other components like protein. It is also of value to preserve native breeds not to lose genetic variation within the species as well as preserving the cultural heritage.

When looking at the milk composition over the whole year it was found that the milk from the SMB and the SRB farms were most alike regarding protein and fat content. Regarding the variation in protein profile, the most influencing factor has been found to be genetic (Dalgleish 1993). In our study, genetic variants are not included as a part of the results. In future research it would be interesting to see an extensive mapping of the genetic variants found in milk from Swedish native breeds such as SMB, in comparison to more frequently used dairy breeds. It would also be interesting to evaluate milk from cows kept on pasture for the majority of the summer, to be able to map out the potential effects of pasture on the milk protein profile with more accuracy.

6 Conclusion

When investigating the protein composition in milk from Swedish Mountain Breed, Swedish Jersey, Swedish Red and White and Swedish Holstein cows, some differences were found. Swedish Jersey and Swedish Mountain Breed milk was found to include the highest concentration of κ-CN, which was the protein fraction that varied most between the breeds, both when comparing annually and between periods. The concentrations of total protein, total CN and total whey were stable over the year. Season was not found to influence the protein composition to any significant extent. During the pasture period, much of the feeding was done indoors, which might have been a contributing factor to the small changes in the milk protein profile between the periods. For this study, only a small number of farms were included which might affect the obtained statistical results. The results could therefore be considered as implications. More extensive studies would be needed in order to further support the outcomes.

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Acknowledgements

I would like to thank my supervisor Monika Johansson for all the help and support in the lab and throughout the writing process. I would also like to thank my assistant supervisor Hasitha Priyashantha, especially for helping me with the statistical analysis and my other assistant supervisor Gun Bernes for the much valued help during the writing process. Lastly, I would like to thank my friends and family for believing in me and helping me to cross the finish line.